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Research report

Differential navigational strategies during spatial learning in a new modified version of the Oasis maze

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ABSTRACT

During spatial navigation, some typical parameters of learning have been observed, such as latency or path length. However, these parameters are sensitive to patterns of navigation and orientation that are not easily measurable. In the present study, we used a modified version of the Oasis maze and evaluated different parameters of learning, navigation, and orientation in different animal groups. Through a PCA (Principal component analysis) we found different factors such as learning, navigation, speediness, anxiety, orientation, path variability, and turning behavior. Each factor gathers different groups of behavioral variables. ANOVA analysis of those factors demonstrates that some of them are more strongly modulated by trial progression, while others by animal group differences, indicating that each group of variables is better reflecting one of these dimensions. To understand the nature of these navigation differences, we studied orientation strategies between animal conditions and across trials. We found that the main navigational strategy used by the animals consist of locating the target and directing their behaviors towards this area. When testing how this strategy changed after cognitive impairment or enhancement, we found that AβOs treated animals (Amyloid β Oligomers, Alzheimer animal model) have strong orientation difficulties at locating the target at longer distances. While animals with learning enhancement (exercised rat) do not show changes in orientation behaviors. These analyses highlight that experimental manipulations affect learning, but also induced changes in the navigational strategies. We concluded that both dimensions can explain the differences observed in typical learning variables, such as latency or path length, motivating the development of new tools that asses this two-dimension as a separate but, interacting phenomenon.

1. Introduction

The hippocampus is a crucial structure associated to spatial learning [1,2], many studies had used different behavioral task to asses spatial learning, in rodents and humans. Among these are the Morris water maze [3], Barnes maze [4], annular water maze [5], Oasis maze [6,7], ziggurat [8], multiple T-maze [9], Y-maze [10] between others [11]. In humans, virtual environment has been used to test spatial navigation learning, such as the Virtual Morris water maze [12], the yellow cab [13] between others [14]. Despite the variety of existing tasks, only a small number of behavioral parameters has been used consistently to evaluate spatial learning performance. The most frequently used parameters have been latency or path length, even though spatial navigation contains many different parameters susceptible to be analyzed that could improve the understanding of the whole process. Some examples are turning angle, time spent in the center or periphery of the maze, straightness (ratio between observed and the shortest path), and entropy (variability point to point of the navigated path) [15–17]. However, is still unclear which of these parameters are better associated with learning, which of them reveal navigation or spatial orientation strategies, or even which are correlated with other variables such as anxiety [18], or with intrinsic factor of animals or experimental manipulations [19]. For these reasons the purpose of this work is to comprehensively describe the changes in navigation that occur during spatial learning and to determine which set of navigation parameters could better describe changes in spatial navigation and learning., e.g.

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which parameters are more sensitive for learning across trials or which are more sensitive to navigational performance between different animal groups or experimental settings. In the same way, simple variables such as latency could be explained by changes in other behavioral parameters such as velocity, orientation times or orientation distance, which gives a better comprehension of why the learning progression in some animals is most efficient than in others.

To convey these problems different approaches have been previously conducted, mainly using different types of factor analysis. The principal challenges of these approaches are determining the best methods to group different behavioral parameters as a single underlying variable and then after the variables are identified, to clearly interpret them. For example, [20]) analyzed several path variables of Hemi-cerebellectomized animals solving the Morris water maze [21]. They approach was to use an automatic method to classify different spatial navigation strategies into several pre-defined categories that represent typical behavior on the water maze, as thigmotaxic (when animals stay close to the walls of the maze) or circling. This method allowed to select among all variables, those that are associated with a navigational strategy, facilitating the interpretation of the grouped factors. Nevertheless, in this study, the authors did not associate these factors with changes in learning, or with differences between animal conditions, and the analysis was biased by the a priori selection of representative navigation types.

In the same way but using a different methodological approach Wolfer et al. [22,22] studied a highly variable animal population of genetically modified animals, using factor analysis. They identified four latent factors that were interpreted as different navigational behaviors, such as thigmotaxic and passivity. However, in this work, the factors were not evaluated regarding how they changed across trials or animal groups. For this reason, the authors concluded that those factors are not necessarily associated with cognitive strategies, and then the behavioral changes observed must be interpreted carefully, since other behavioral components, not directly related to learning and memory, may explain the differences. In fact, other authors [16,19] have shown that some variables, as lingering time or distance traveled, were mainly explained due to animal characteristics or even the laboratory where the experiments were done.

Considering that the grouping of proper variables and the factor interpretation are two coexisting difficulties when analyzing behavioral performance through factor analysis, the present work aims to overcome these problems doing both factor analysis [22] and then testing the changes of the obtained factors across trials and groups. To solve this issue, it is necessary a high behavioral variability, with different animal groups with expected enhancement or impairment on spatial learning, which able us to better interpret our factors.

We develop a new modified version of the Oasis maze [23], less stressful, where the reward was used as the main driving to solve the task, instead of water or bright light like in the Morris water maze or Barnes maze, with no animal handling during task execution. Four experimental groups were used, sedentary/exercised rats (learning enhancing) and AβOs/Saline (learning impairment), to aim a wide variability in animal spatial learning capacities. PCA, ANOVA and linear model analyses between experimental groups reveal several parameters related to navigational strategies explaining differences between groups and learning progression across trials. Further analysis to better describes these changes in navigation between groups and across trials indicated that they could be explained as differences in animal capacities to get orientate at longer distances.

2. Material and methods

2.1. Animals

Thirty-four male adult Sprague Dawley rats, weighing 270 − 330 g, were obtained from the institutional animal facility. Rats were maintained under 12/12 light-dark cycles, ZT0 at 07:00 a.m., individually caged in a temperate room (23 °C) under food and water *ad libitum* schedule, until other condition will be indicated. All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and local institutional Bio-Safety and Ethical Committee (CBA #0755; CBA#0337 FMUCH) minimizing the number of rats used and their suffering. Two sets of animals were used. One of them to prompt an increase in cognitive abilities by exercise [24] and another one with a cognitive impairment induces by Amyloid β oligomers (AβOs) intrahippocampal injections [23]. The first set was divided into two groups: 1) voluntary exercise animals ("exercise" group) which were individually housed during 21 days with free access to a running wheel of 25 cm of diameter and 10 cm of width, that was enabled with an electromagnetic wheel spinning counter to determine the total distance run by the animals daily (n = 11); and 2) "sedentary" animals ("sedentary" group), that were maintained in the same housing condition but without a running wheel (n = 11).

The second set of animals were chronically implanted with injections cannula targeting the CA3 region of the dorsal hippocampus, bilaterally (see section surgery for details). This set of animals was divided into two groups, the "AβOs" and "saline" groups. After surgery recovery, AβOs animals received three sequential bilateral injections of 0.5 µl of AβOs (Aβ1-42 peptide, n = 6) or saline (n = 6) in a 48 h period as has been described previously [23]. The two sets of animals were tested during six consecutive days in the Oasis maze task described below. The AβOs and the saline group were tested five days after first injections, and the exercise and sedentary groups after 21 days in the appropriate housing condition. These AβOs/saline animals were used before for another unrelated study [23] were cannula position in the hippocampus was previously confirmed.

2.2. Maze and protocol

To test spatial navigation learning, we used a modified version of the Oasis maze [6,7,25] it is a dry-land version of the Morris water maze equivalent in hippocampus spatial navigation requirements. The apparatus consisted of an open field arena of 1.4 m diameter, at 50 cm from the floor with a wall of 20 cm of height, located in an isolated room with constant distal visual cues. Twenty-one evenly spaced tight to the board wells (4.5 cm diameter, 2 cm height) were positioned on the board, and one of the wells was baited with water (Fig. 1A). The task consisted of 2 steps: first, during the "pre-training" phase, the rats were water-deprived by 24 h and pre-trained to seek a water drop inside of the wells, during 3 consecutive days, up to the animals was able to find all the rewards in 10 randomly distributed baited wells before 10 min of exploration was elapsed. The next step (testing) consisted of 15 trials of 1 min each per session, one session per day, during 4–6 consecutive days. During each trial, the rat was enclosed with a black cylinder of 22 cm in diameter and 27 cm in height over the arena. The trials started after the cylinder was removed, and it was ended when the rat reached the reward or 1 min was elapsed. During 20–30 seconds of the intertrial period, the animal was again enclosed with the cylinder and gently moved to a different starting position randomly and counterbalanced through each trial. This strategy reduces the handling and potential stress of the animal trial to trial and prevents the stereotyped trajectory of the animal to solve the maze when the starting point is always the same. During each testing day, the reward was changed to a new position, to promote new spatial learning.

2.3. Surgery

Regarding the AβOs and saline groups, rats were anesthetized with isoflurane (2.5 % in oxygen) for induction and 1.5 % for maintenance, at 1 L/min of oxygen flow. Sedation depth was monitored by the absence of a toe pinch withdraw reflex. The animal was head restrained

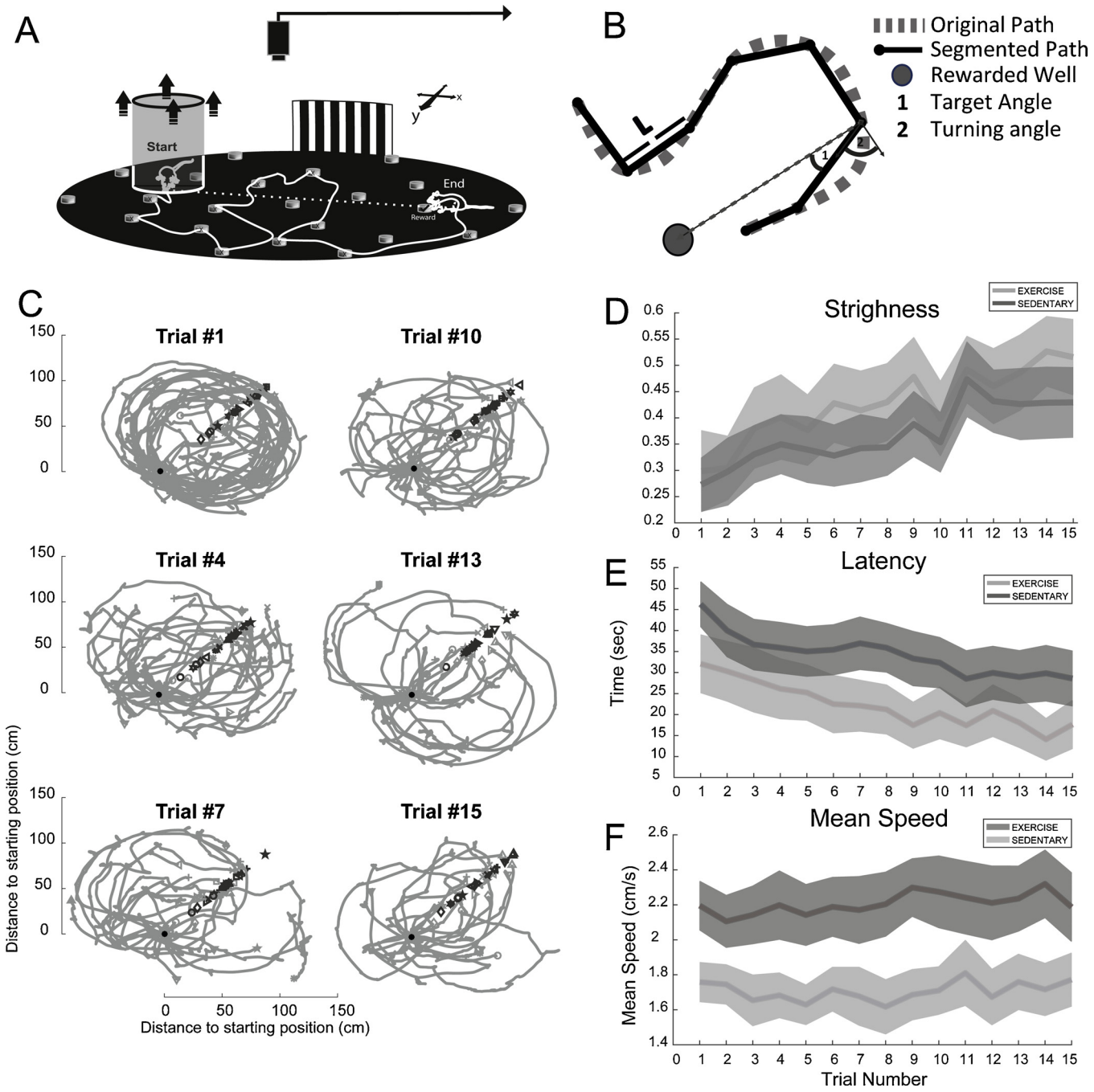

Fig. 1. Behavioral Paradigm and trial progression. A) Task Scheme. Twenty-one wells were equidistantly disposed over a circular arena, were one of them was baited with water. Rat start the task at a random position once the cylinder is removed. A proximal visual cue was located at one of the sides of the arena. Behavior was video-recorded from the zenithal position, which allowed the reconstruction of the trajectory (white line). B) Segmentation procedure and angular definitions. Segmentation is generated by fitting segments of the same length "L" along the original path. Two angles were defined. Target angle (1) which is comprised between the current direction of the animal and the vector between the current animal position and the rewarded well. Turning angle (2) which is the angle comprised between two consecutive directions in the animal path. C) Representative progression of paths through trials. For each trial (1, 4, 7, 10, 13, 15) paths of 2 sessions of 8 different rats were plotted together, by centering the starting position of the animal at the origin (x = 0, y = 0), and by rotating the trajectories by an angle such that the rewarded well remained on the diagonal (x = y, 45°). This sequence of paths depicts changes in orientation strategies through trials. D) Mean and standard deviation of straightness coefficient through trials for exercise (light gray) and sedentary (dark gray) groups. The bold line represents mean value across rats, and the shaded region the standard deviation. E) and F) same as D), but for Latency and Mean Speed.

with a stereotaxic frame, an incision on the skin and a small craniotomy was conducted to implant two bilateral stainless-steel cannula guides of 26-gauge (Plastics One), targeting the dorsal CA3 region of the hippocampus following stereotaxic coordinates according to the rat brain atlas [26] (AP 2.5 mm; L ± 3.5 mm. and 2,7 mm depth). Cannulas were fixed to the skull with anchors jewelry stainless steel screws and dental acrylic. Antibiotic (Enrofloxacin, 19 mg/kg i.p.; Bayer) and anti-inflammatory (Ketophen 0.2 mg/kg i.p.; Rhodia Merieux) were administered at the end of surgery and during three consecutive days.

Table 1
Variable description.

Variable name	Definition	PCA Variable
Speed	Length of the velocity vector. Correspond to the absolute value of the polar coordinates of the velocity vector	Mean and STD
Movement Direction	Polar angle of the Velocity vector	Mean and STD
Distance to Objective	Defined as the distance from the animal to the Target, at each position	Mean and STD
Angle to Objective	Angle to Objective was defined to assess any bias to the target during the path. It is defined as the angle comprised between the velocity vector and the vector between the animal current position and the target. Mean and STD are estimated using angular statistics.	Mean and STD
Turning Angle	The angular difference between two consecutive turns	Mean and STD
Angle to Center	The angle of the polar coordinates of the position of the animal respect to the maze center.	Mean and STD
Step correction correlation	Correlation between the turning angle and the target angle.	Single Value
Acceleration	Length of the vector resulting from the difference between two consecutive velocity vectors	Mean and STD
Meander	Quotient between turning angle and speed.	Mean and STD
Nsteps	Number of 113 cm steps made by the rat (assuming fixed stride size along the trajectory).	Single Value
Orientation Distance	Defined as the distance between the animal position and the target at the time of the Orientation Time (see below)	Single Value
Distance to Center	The distance of the rat to the center of the maze.	Mean and STD
Distance to Border	Defined as the distance from the animal to the border of the maze.	Mean and STD
Border and Center Time	Time spent at 50 cm or less from the border or center of the maze.	Single Value
Orientation Time (angular, distance)	Defined either as the time after that all the angles to the target are lower than 90° (angular) or at which the position of the animal remains within a circle of 50 cm of diameter from the target (distance).	Single Value (each)
Angle to Objective (90°)	Same as Angle to the objective but shifted 90°. This variable can differentiate between random angular values from angles centered in the target direction. Under this definition a random angular value would result in a value of 0, but angles centered in the target would average closest to 90°.	Mean and STD
Normalized Orientation Distance	Orientation Distance normalized by the Euclidean distance between the starting position of the rat and the target.	Single Value
Orientation Time (mixed)	Defined as the time at which the product between the target angle and the distance is monotonically decreasing	Single Value
Straightness	Defined as the ratio between de Euclidean distance to the target from the starting position of the rats and the observed path length.	Single Value
Path Entropy	It is defined by Maei (2009), as the natural logarithm of the product between the variance of each coordinate of the animal trajectory. $E_E = \ln(\sigma_x \sigma_y)$	Single Value
Error Entropy	Is the natural logarithm of the square of the variance of the distance to the target. $E_P = \ln(\sigma_T^2)$	Single Value
Pathlength	Defined as the length of the animal path.	Single Value
Latency	Duration of the trial.	Single Value
Success	Defined as 1 when the animal finds the target and 0 otherwise	Single Value

Each row contains the variables included in the PCA. The first column depicts the variable name. The second column a brief description of the variable. The third column indicates if the mean and standard deviation was included on the PCA. When the variable depicts a general feature of the trajectory (as correlation value or orientation time), "Single Value" is indicated on the same column.

2.4. Video recording and tracking

All the animal behavior was video-recorded, with the help of a video camera in a zenithal position. Videos were recorded at 30 frames per second, with each frame being an uncompressed image of 240 × 320 pixels. The maze and wells were black to produce good contrast between the albino rat and the maze, to facilitate the offline video tracking. With custom made Matlab routine, the position of the animal was traced at each frame, and a reconstruction of the navigation was built. Also, the position of the center of the maze, of every well and the distance between the center and the border of the maze was obtained in each video to compare the trajectory of each animal between trials properly.

2.5. Variables

Once the trajectory of the animal was obtained, several navigation parameters were estimated, such as entropy [17], latency and path length [3,27], straightness [15] and angular orientation, among others (see Table 1). They were selected according to previous reports on similar tasks, like Morris Water Maze and Open Field [19,20,22] with the aim to determine which of these parameters evolve during learning and which of them are better descriptors of spatial navigation. Also, we determine which of these parameters is better to discriminate intergroup differences. The description of all variables is depicted in Table 1.

2.6. Data analysis and statistics

All analyses were executed in Matlab (Math Works, Inc), excepting the factor analysis, which was done under RStudio (RStudio Team 2015). The trajectories were preprocessed by smoothing the path to avoid artifacts introduced by the tracking routine. We used the "Lowess" form of the Matlab smooth function, with a span of 15 frames, that is, of 0.5 s, similar to other reports [28]. This procedure has little effect on the spatial accuracy of trajectories while removing most of the velocity artifacts introduced by the tracking system. In total, 2333 trials were analyzed, 667 from the exercise group, 680 from the sedentary group, 536 from the AβOs group, and 450 for the saline group.

2.7. Factor analysis

Factor Analysis was made on RStudio RStudio Team 2015. The data analyzed was a 36 × 2333 matrix, of 36 variables listed in Table 1 and 2333 observations, comprising the four groups under study. The *Step Correlation* variable needed a minimum number of points to reach interpretable values, then 47 trials were not included in this analysis (further details on step correlation section), resulting in a total of 2286 trials for the factor analysis. The analysis was made following two steps: 1 determining the number of latent factors and 2 performing the decomposition in the number of factors chosen. To select the proper number of factors we used optimal coordinates as suggested in [29]. The factor decomposition was made using the principal axes method as several variables were not normally distributed. The Oblimin rotation was preferred, as is expected that factor may correlate to some extent, and orthogonal rotation gave similar results (Supplementary Table 1).

Once the principal factors were obtained, a two-way ANOVA was performed for each principal component using group and trial number as the two factors, to determine the dependence of each factor with these two variables. To estimate the percentage of the total variance explained (or effect size) for each factor, we calculated partial-η_{factor}

[30] as defined by the formula:

$$\text{partial}-\eta^2_{factor} = \frac{SS_{factor}}{SS_{factor} + SS_{error}}$$

Where SS is the sum of squares from ANOVA for the factor and the error, when needed, linear models were estimated to assess linear relation among factors and trials. For *post hoc* test *t*-test corrected for multiple comparisons (Bonferroni corrected) were performed, either comparing all group or trial combinations. When a trial was significantly different from several trials, the higher p-value was reported.

2.8. Navigation assessing

To assess an animal's navigation strategy, we performed two analyses: angular correlation analysis and angle v/s distance analysis.

2.8.1. Angular correlation analysis

To perform the angular correlation analysis, the trajectories were segmented in steps of the same length "L," to generate a discretized trajectory. The procedure is described in [31]. Briefly, assuming a step length L, the first point of the discretized trajectory is chosen as the first point of the real trajectory. The second point in the segmentation is the first point of the remaining real path that is L cm away from the starting position. The procedure continues choosing the next discretized point as the first point of the remaining real trajectories that are at L cm of the distance of the last discretized position. The procedure continues until no point at L distance of the real trajectory can be found. If the last point of the real trajectory is at least half of the step "L" from the previous discretized point, it was included to avoid losing the last portion of the trajectories. The rational under this segmentation is that fixed-step segmentation would better represent the actual strides done by the animal, instead of the arbitrary size imposed by the video sampling rate. We first used a step length of 11 cm as was previously estimated [32], which correspond to an average rat stride length, and then compared this result by using different step lengths.

The step correlation was computed using the discretized trajectories, by calculating the Spearman correlation between a target angle and the consecutive turning angle. For this purpose, we calculated the angle to the target and the turning angle (Fig. 1B). The target angle was estimated as the angle comprised of two vectors: The first vector is defined between the current animal position and the target and the second vector, between the current animal position and the position of the animal in the next vertex of the segmented trajectory. The turning angle is defined as the angle between the change in directions of two following animal steps. Then, if the rats correct their trajectories in the direction of the target both values (angle to the target and turning angle) must be correlated. That is, in the case the rat can locate the target, if the angle between the rat direction and the target is large, then the turning angle should be large too, to correct its trajectory.

Conversely, if the rats are walking with a slight angular deviation to the target, then the turning angle value should be small to continue moving towards the target. That is means that if rats can locate the target, then the correlation values must be positive. If rats do not correct their trajectories, the turning angle may not vary after large deviation to the target, and then the correlation value should drop to zero.

This procedure gives a single correlational value for every trajectory and predicts correlation values closer to 1 for oriented trajectories, and near to 0 for disoriented animals. The histogram of correlations values is shown at different trial numbers. A one-way ANOVA with a trial number as a factor was performed, and the corresponding *t*-test as post hoc. We then calculated the correlation between the trial number and the step correlation value to asses any improvement in the orientation strategy. We also used this analysis to compare different step lengths. Specifically, we expect that the real 11 cm step length will maximize the correlation between trial and step correlation values, as should be reflecting the actual strategy of the animal. For this purpose, the same step correlation procedure was repeated varying the value of "L" from 1 to 30 cm at one cm steps, and then the Spearman and Pearson correlations were calculated between trials and step correlations, for each step value.

2.8.2. Angle v/s distance analysis

Angle v/s distance analysis aimed to detect any navigational bias to the target zone. First, every trajectory was included in the analysis, and then the same analysis was performed per rat. For every point in the animal trajectory several values were calculated, the angle to the target, the distance to the target and the distance and the angle to the other non-rewarded wells. Target angle was estimated as the angle comprised of two vectors: The first was between the current animal position and the target and the second vector was between the current animal position and the position of the animal in the next sample, 0.03 s later on the real trajectory (in analogy with the angle estimated on the step correlation analysis). This angle is minimum at 0°, when the animal is walking directly in the direction to the target and maximal at +/-180° when the animal is walking in the opposite direction. The target distance was calculated as the Euclidean distance between the animal position and the target position. The same procedure was repeated for the other 20 non-rewarded wells. This analysis resulted in 21 angular values and 21 distances to wells where one was the target, the rewarded well. In total, 1,671,778 values were analyzed, when all 2333 trajectories were included.

For plotting the angle -distance variables distribution, angles, and distances were binned at 1° and 1 cm respectively. Values of distance to the wells lower than 5 cm were not included, because when the tracking system artificially located the animal above the wells, it generated artificially near 0 angular and distance values. As the analysis aimed to detect navigational biases in direction to the wells, removing those data where the animal position is above the wells would not contribute to the analysis previously described.

Angle to the wells variance and mean were estimated for target and non-target conditions, using the Circular Statistic toolbox [33]. Briefly, the mean and variance of a set of angles were calculated averaging the set of unitary vectors generated from those angles. Thus, the mean is estimated as the angle of the resulting vector; and the variance as one minus the vector length.

To estimate the navigational bias, we first built angular bins by dividing the distance to the target into 5 cm windows from 5 to 120 cm. The bins comprised all angles associated with distances falling within the corresponding range. This procedure generated 23 five-centimeters ranges. We calculated the angular variances and mean at each bin, as was described in the previous paragraph regarding circular statistics. This procedure gives 23 measures of variances and means, one for each distance range. The same analysis was performed for rewarded and non-rewarded wells angles, on each trial and rat.

Slopes and correlations were estimated using the variances calculated as described above, for each rat, trial, and target and non-rewarded wells. This binning-averaging procedure gave us 30 variance-distance curves, 15 for each well condition, where each one describes the relation of angles and distances of a rat in a trial. An example of these curves can be found in Supplementary Fig. 1. They represent the ratio of change of the target angle as a function of the distance to the target. Positive slopes mean that rats tend to have smaller target angles at a closer distance, and steeper slopes are interpreted as stronger orientation bias toward the rewarded well. We first performed ANOVA and *post hoc* analysis of these data points, to determine interactions between angular variances, and distances, at different trials and well conditions to reveal differences in the target angles between rewarded and non-rewarded wells. In a secondary analysis, using these same curves, we calculated the slope of the fitted linear model (using least squares) and the Spearman correlation for each curve, to study any change on the orientation's bias (represented by the slopes) along with

the trials. This analysis resulted in 34 slopes and correlations per trial and well type (one for each rat). ANOVA and *post hoc* analyses were performed using both parameters, trial (1–15) and well type (rewarded and non-rewarded) as factors.

2.8.3. Orientation probability analysis

To estimate the animal probability of getting oriented at a given distance to the rewarded well, we pooled all animal trajectories by trial. We then estimated the former probability as the ratio between the number of times an animal found the target at a fixed distance, among all the times the animal was at this distance from the target. We counted as "finding the target" the events in which the animal found the reward within 4 s after visiting a given location. We repeated the same analysis changing the length to the time window between 3 and 10 s, and similar results were obtained.

3. Results

3.1. Factor analysis

Animals performed 4–6 sessions, one per day and using a different rewarded well each day. Each session had 15 trials with one rewarded well at the same location on every trial. To visualize the changes in trajectories across trials, a random subset of trajectories from different sessions and animals were plotted together throughout progressive trials (16 trajectories, in 2 sessions of 8 rats, Fig. 1C). Trajectories starting point were fixed at the origin of a reference cartesian plane (black dot), and the trajectory orientation was rotated until the target of the same trial were located at 45 degrees from the x/y axis of the reference plane (different markers at the end of each trajectory), since every rat or session has different rewarded well locations. It is possible to observe how the trajectories are changing across trials with straighter paths and shorter latencies, as can be observed on Fig. 1D, E, for sedentary and exercises rats.

We found several variables that change along with trials such as latency or straightness, while others only show significant changes between groups, as Mean Speed in exercise and sedentary groups (Fig. 1F). Because some navigational parameters such as speed may have an effect over learning parameters such as latency, we perform PCA analysis to separate the contributions of each of these processes.

The optimal coordinates analysis resulted in 7 factors which can be observed in Table 2. They explain 58 % of the data variability. Even though Varimax rotation, increased this percentage to 68 %, the data presented here uses the Oblimin rotation, to obtain better-fitted factors and without a significant loss of explained variance. The Kolmogorov test showed that each factor was normally distributed ($p < 0.00001$, alpha = 0.01, Bonferroni corrected). The ANOVA and linear regression analysis among these seven factors showed a significant correlation for learning progression, group differences or both (see Table 2 and Supplementary Fig. 3). The factors which showed the biggest effect on learning were factor 1 and 3 (learning and speediness, partial $\eta_t = 0.148$ and partial $\eta_t = 0.144$) and the factor which showed the biggest effect on groups differences was factor 2 (angular variability partial $\eta_g = 0.239$). Some factor such as factor 5 (orientation partial $\eta_t = 0.01$; partial $\eta_g = 0.002$); and factor 7 (partial $\eta_t = 0.005$; partial $\eta_g = 0.0004$) were not correlated with any of these dimensions.

The factor more strongly related to learning progression include variables which are typically described in the literature, such a latency, straightness, and path length as can advise on factor 1. The second factor correlated with learning progression was speediness and include the variables speed, path entropy and meander (among others). The factor with the stronger effect over group differences was the second factor (angular variability) which includes variables such as angle to the target, turning angle and distance to the border, but not latency or path length, suggesting that different groups of variables describe learning progression and animal groups differences. Since speed and latency are not contained in the same factor, these two variables could evolve partly independently, and then the interpretation of latency changes must be careful since faster animals would have shorter latencies. In addition, the fourth factor (anxiety level) which includes variables such as distance to the center, distance to border and central time indicated differences among groups but not learning progression. These results suggest that differences between groups may emerge due different navigation strategies, which may be indirectly detected by these sets of parameters. In the following section we develop some complementary analysis to detect these navigational differences more directly.

3.2. Navigation strategies

Now that the relevant variables reflecting the learning progression and animal group differences have been identified, it remains to assess the navigation strategies that lead the animals to get orientated. Different possibilities can explain the progression in learning during this task, such as animal's residual olfactory cues or procedural learning strategies, among others. As can be expected in this kind of spatial learning task a strong orientation guided behavior must be observed. Also, different navigational strategies could be observed in different animal groups. To test this idea in the next section, we describe the strategy by which rats direct their trajectories to the target, trough trials, and different animal groups, validating the interpretation of the previous factor as measuring spatial orientation and learning.

3.3. Orientation strategy

To assess the orientation strategy, we estimated the capacity of the rat to direct their trajectories to the target. To this purpose, we calculated the correlation between the turning angle and the target angle (see Methods for more detail). Under the assumption that rats can redirect their path toward the target step by step, the correlation between these two angles should be positive, otherwise, the correlation must be close to zero. Under this assumption, we expected low values of correlations during the first trials and high correlation values for the latter ones.

As expected, correlation values show an increase across trials (Fig. 2A). During the first trial, the peak of the step correlation distribution is centered around 0.4, while for the last trials the peak is centered around 0.8. The Spearman correlation between trial number and step correlation value was significantly and positive ($r = 0.1004$, p-value = 1.4830e-06). When testing the same analysis for different stride lengths, the best combination of high correlation values paralleled by a lower p-value is obtained for a stride length of 6 cm, followed by the 11 cm, which is the same stride length estimated for rats in a previous report [32], Fig. 2B.

3.4. Target identification

The previous measure gives a global approach to assess the orientation strategy used by rats to solve the task. Nevertheless, it does not measure the current orientating process occurring within each trial. To solve this issue, we analyzed the rat's angle to the target according to its distance. If the previous analysis of step correlations is correct, then within each trial the angle to the target should decrease as the rats approach it.

Additionally, this relationship should not be present for non-rewarded wells. In Fig. 3A (upper panel) a density plot of angle and distance to the target is depicted, where the estimated probability of finding a combination of distance and angle to the target is shown. We can observe an accumulation of small angles and distances to the target values (Fig. 3A, upper panels on the left). This combination of small angles for short target distances is not present when doing the same density plot for non-rewarded wells (Fig. 3A, upper panels on the right).

Table 2
Summary of Factor Analysis.

#	Name	Variable included	Trial progression	Group difference	% Var
1	Learning	Angle to Center (STD) Target Time Orientation Time (angular) Orientation Time (distance) Orientation Time (mixed) Straightness Path Length Latency Number of Steps Success ratio.	ANOVA F = 27.71 p < 6.68e-68 Effect size partial-ηt = 0.149 Linear model F = 213, p < 3.04e-46	ANOVA F = 49.06 p < 1.07e-30 Effect size partial-ηg = 0.062	18
2	Angular variability	Angle to Target (STD) Turning Angle (STD) Meander (STD) Angle to Target 90° (STD) Speed (mean) Speed (STD) Distance to Border (STD) Distance to Target (STD) Straightness	ANOVA F = 6.41 p < 8.05e-13 Effect size partial-ηt = 0.039 Linear model F = 58, p < 3.91e-14	ANOVA F = 232.94 p < 1.95e-131 Effect size partial-ηg = 0.239	11
3	Speediness	Meander (STD) Movement Direction (STD) Speed (mean) Acceleration (mean) Path Entropy Number of Steps Linear model n.s.	ANOVA F = 26.79 p < 1.39e-65 Effect size partial-ηt = 0.144	ANOVA F = 7.7 p < 4.04e-05 Effect size partial-ηg = 0.010	9
4	Anxiety level	Distance to Center (mean) Distance to Center (STD) Distance to Border (mean) Center Time	ANOVA n.s Effect size partial-ηt = 0.0122 Linear model n.s	ANOVA F = 37.06, p < 2.28e-23 Effect size partial-ηg = 0.0476	7
5	Orientation	Angle to Center (STD) Angle to Target 90 (mean) Distance to Target (mean) Orientation Distance Norm. Success ratio Orientation Distance	ANOVA n.s Effect size partial-ηt = 0.010 Linear model n.s.	ANOVA n.s. Effect size partial-ηg = 0.002	5
6	Path variability	Speed (STD) Distance to the border (STD) Distance to objective (mean) Distance to objective (STD) Error Entropy	ANOVA F = 5.18, p < 9.93e-10 Effect size partial-ηt = 0. 0316 Linear model n.s	ANOVA F = 20.8, p < 2.64e-13 Effect size partial-ηg = 0.0273	4
7	turning behavior	Turning angle (mean) Meander (mean)	ANOVA n.s Effect size partial-ηt = 0.005 Linear model n.s	ANOVA n.s Effect size partial-ηg = 0,0004	4

The first column depicts the corresponding factor, the second column indicates the behavioral variables contain on each factor. The third column indicates if the factor detects learning progression across trials (ANOVA, linear model and effect size are indicated). The fourth column indicated if the factor detects the difference between animal groups (ANOVA and effect size is indicated) and the final column indicated the percentage of the total variance represented by each factor.

That means, when the animals are closer to the rewarded well, they are genuinely orienting to that location. Meanwhile, when the animals are closer to a non-rewarded well, they are not orienting to this spatial location.

Even though the highest difference between rewarded and non-rewarded wells is at short distances and small angles, it is still possible to observe a relationship between these two variables at a longer distance. In effect, the angular variance along distances shows a slight slope that starts at around 60 cm but only for rewarded wells (Fig. 3A, bottom, solid line). As we could observe, the angular mean along well distances (Fig. 3A, bottom, dashed line), is continuously around 0 for all distances, either for rewarded or non-rewarded wells. This result is consistent with both random and oriented conditions, because, when angles are uniformly distributed, the average is 0, but also if they are symmetrically distributed around 0. These variables able us to discriminated against when the animals are getting oriented to the target-well, even for more considerable distances. For this reason, since the variance accurately represents the animal orientation, further analysis

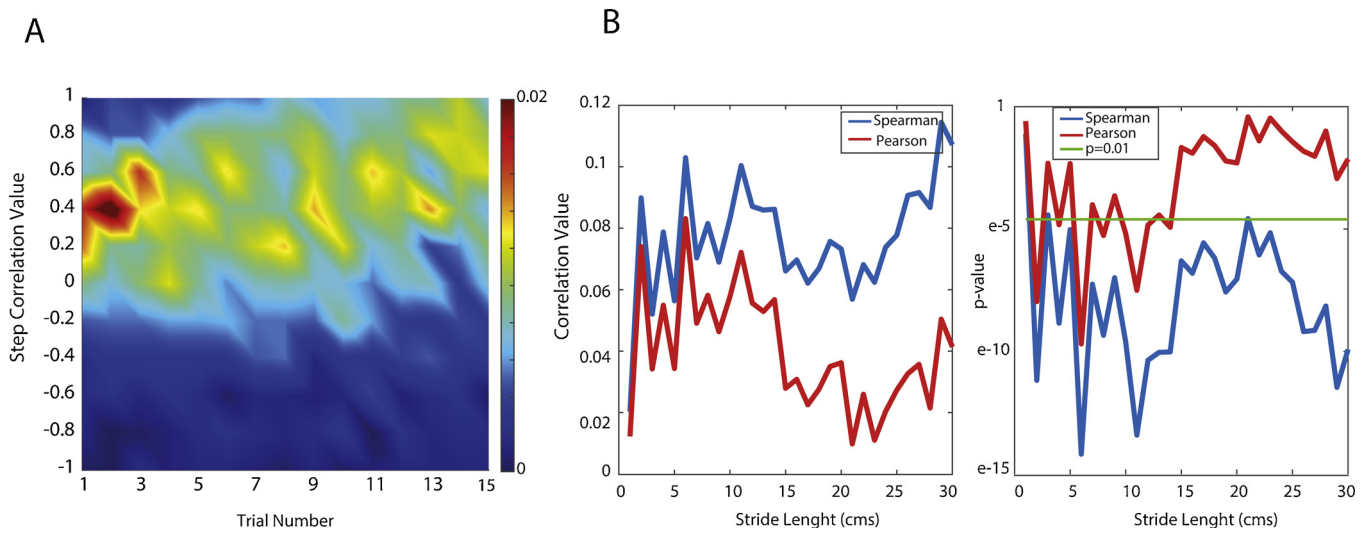

Fig. 2. Step correlation analysis. A) Density plot of the step correlation value and trial number. Color represents the density of step correlation in relation to the total number of step correlation values. The Spearman correlation values fos this data was r = 0.1004. B) Left Panel: Spearman(blue) and Pearson (red) correlations between step correlation values and trial number with respect to the stride length used in the segmentation procedures. The maximum correlation values were obtained with a stride length of 6 and 12. Right panel: p-values associated with each of the Spearman and Pearson correlation. P-value equals to 0.01 is indicated with a horizontal green line.

will be conducted by using only this parameter.

To study the angle to distance relationship for rewarded and non-rewarded wells, we plotted the angular variance along with target distances, for each rat, trial and well type (Fig. 3B). As in the density plot analysis containing all the rats and trials, there is a clear relationship between angular variance and distance. That is present for rewarded wells (red) but not for non-rewarded wells (blue). Now comparing the relationship between distance and angle trough trials, we could observe that the "slope" of the relation between both variables is augmenting along with trials only for rewarded wells, while for non-rewarded wells it stays unrelated. A two-way ANOVA of the angular variance between rewarded and non-rewarded wells and distance as factors shows that angular variance is related to both of them and shows significant interaction (distance factor: d.f. = 22, F = 143.5, $p \sim 0$ within computer precision; well type factor: d.f. = 1, F = 19.607, $p \sim 0$; distance-well type interaction: d.f. = 22, F = 129.74; $p \sim 0$). This result indicates a difference in the relationship between the variables for rewarded and non-rewarded wells conditions.

Now, to assess the association between angle and distance to rewarded wells across the trials, we first performed a two-way ANOVA of angular variances but now with trial and distance as factors. Again, angular variance depends on trial and distance to targets, with significant interaction (distance factor: d.f. = 22, F = 141.55, $p \sim 0$; trial factor: d.f. = 14, F = 37.17, $p \sim 0$; distance-trial interaction: d.f. = 308, f = 1.24; p = 0.0034). This result indicates a difference between the relationship of the variables across trials, that could be explained by an increase in the slope of this angle-distance relationship, as we can see in Fig. 3B (red lines).

This change in slope may reflect that as rats learn to find the rewarded well, they can recognize the target at greatest distances, and consequently, direct their trajectories toward the target, decreasing their angular value. This observation should implicate more skewed angle-distance curves for later trials. A simple way to represent the skewness change is fitting a linear model along with different trials and estimate the slope of the linear model with angular variance as dependent variable and distance to the rewarded well as an independent variable, for each rat, trial and well type (rewarded or not- rewarded). Fig. 3C (left) shows the slope value for each trial, for rewarded (red) and not-rewarded wells (blue). The non-rewarded slopes were around 0, with minimal variability, for all trials. Moreover, rewarded wells condition slopes were greater and showed a slight increase across trials (Fig. 3C, red line). After this, we performed a two-way ANOVA of the slopes with trials and well type as factors. The slope value depended on trial (d.f. = 14; F = 2.26; $p < 0.005$) and well type (d.f. = 1; F = 455.9; $p \sim 0$), with no interaction (d.f. = 14; F = 0.91; p = 0.5467). We then performed a multiple comparison test, between trials and well type, to determine if the slope mean of the rewarded wells was different across trials. Comparing the first trial again the others show that the 7^{th}, 12^{th} and 14^{th} trials were significant different to the first one (trial 7^{th}: d.f. = 66, t = –3.0877, $p < 0.0413$; trial 12^{th}: d.f. = 66; t = -4.5160, $p < 0.004$; trial 14^{th}: d.f. = 66, t = -3.0762, $p < 0.0427$, Bonferroni corrected). When comparing slopes between rewarded and non-rewarded wells, all trials showed a significant difference (d.f. = 66; t 5.8105 and higher; p = 1.9644e-07 or lower). To corroborate these findings, we performed a similar analysis using correlation values instead of slopes. For this purpose, we compute the Spearman's correlation between distance and angular variance, for each rat, trial and well type. Similarly (Fig. 3C, right panel), correlation values for non-rewarded wells stay around 0, while values for rewarded wells were higher. Again, for each trail, rewarded vs not-rewarded correlations were all significantly different (d.f. = 66; t = 4.2030 or higher; p = 8.0881e-05 or lower). Consistently trials 12 showed a significant difference against first trial (d.f. = 66, t = -0.9040, $p < 0.0031$). These results agree with the idea that the change in the variance of angle respect to the distance occurs in a progressive way across trials and indicates that the animals are orienting more accurately to the rewarded wells as the trials progress. This orienting process will be occurring inside of each trial, consistently with the step correlation analysis.

3.5. Navigation strategies trough groups

The previous results indicated that the angle to the target depends on the distance of the animal to the rewarded well. The slope between these two variables increases across trials, indicating that the animals get orientated at longer distances throughout the progression of the spatial learning task.

Now to determine if these changes in the slope of these two variables could discriminate between animal groups, we conduct the same analysis but for each animal condition. In Fig. 4A the change in the

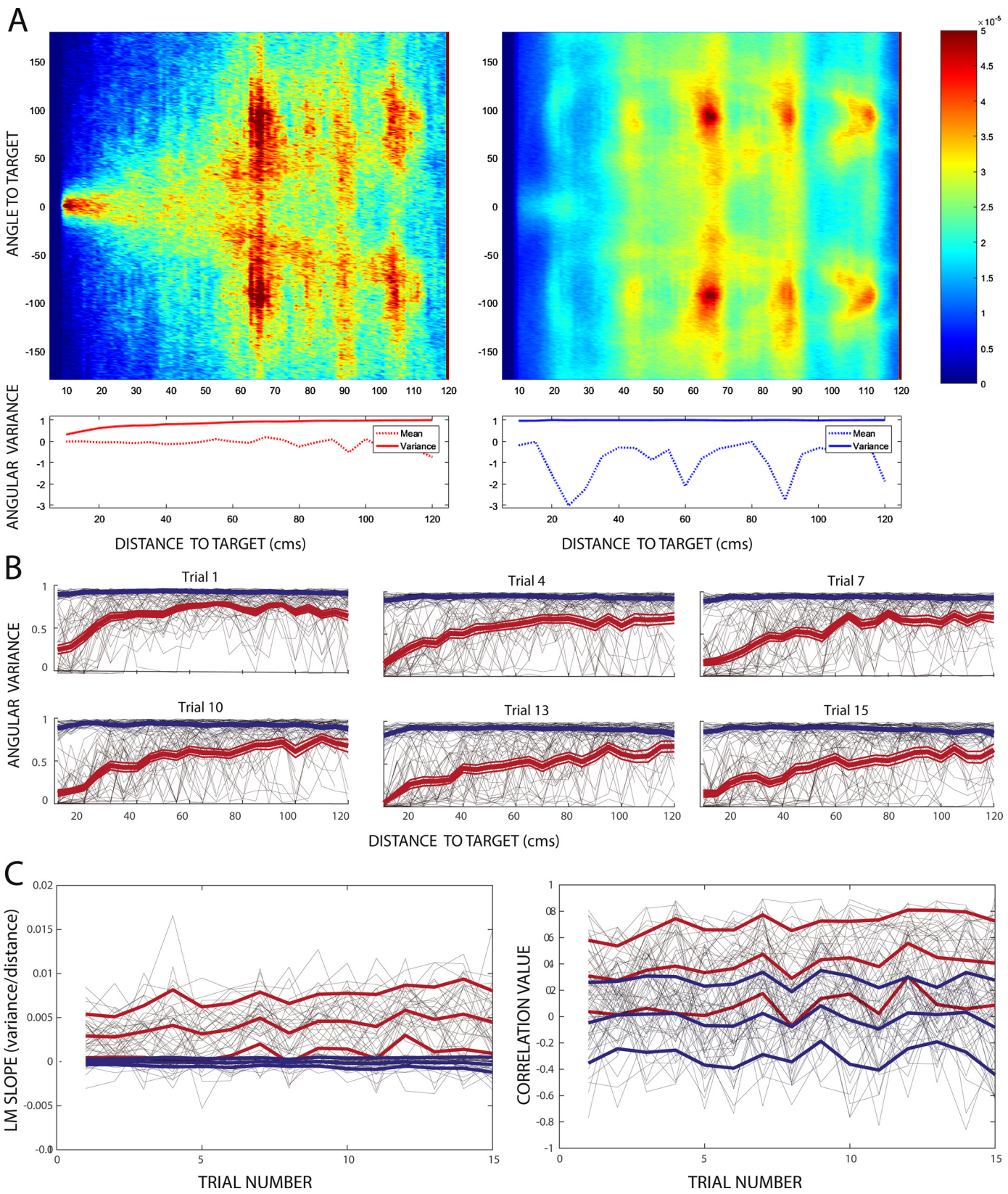

Fig. 3. Angle to Distance analysis. A) Density plot of target angles and distance to target, for rewarded wells (left panel) and for non-rewarded wells (right panel). Color represents the proportion of the number of angle-distance pairs at each bin in relation to the total number of angle-distance pairs. The panels at the bottom indicate the mean angular values and the corresponding variance, for each of the possible target distances (red for rewarded wells, and blue for non-rewarded ones). B) Same as A), but for each rat and trials independently (black lines), blue lines show the mean of and standard deviation of the variances for rewarded (red) and non-rewarded (blue) wells. C) Correlation and slopes of each of the variance curves obtained in B. Left panel: Correlation between angular variance and target distance, per rat and trial. Continuous black lines indicate correlations between angular variance and target distance, for the same rat through trials. Blue lines indicate mean and standard deviation of the correlation values obtained for non-rewarded wells, and the red lines the mean and standard deviation for rewarded ones. Right panel: Same as left panel, but for the slope of the corresponding linear models instead of the correlation value.

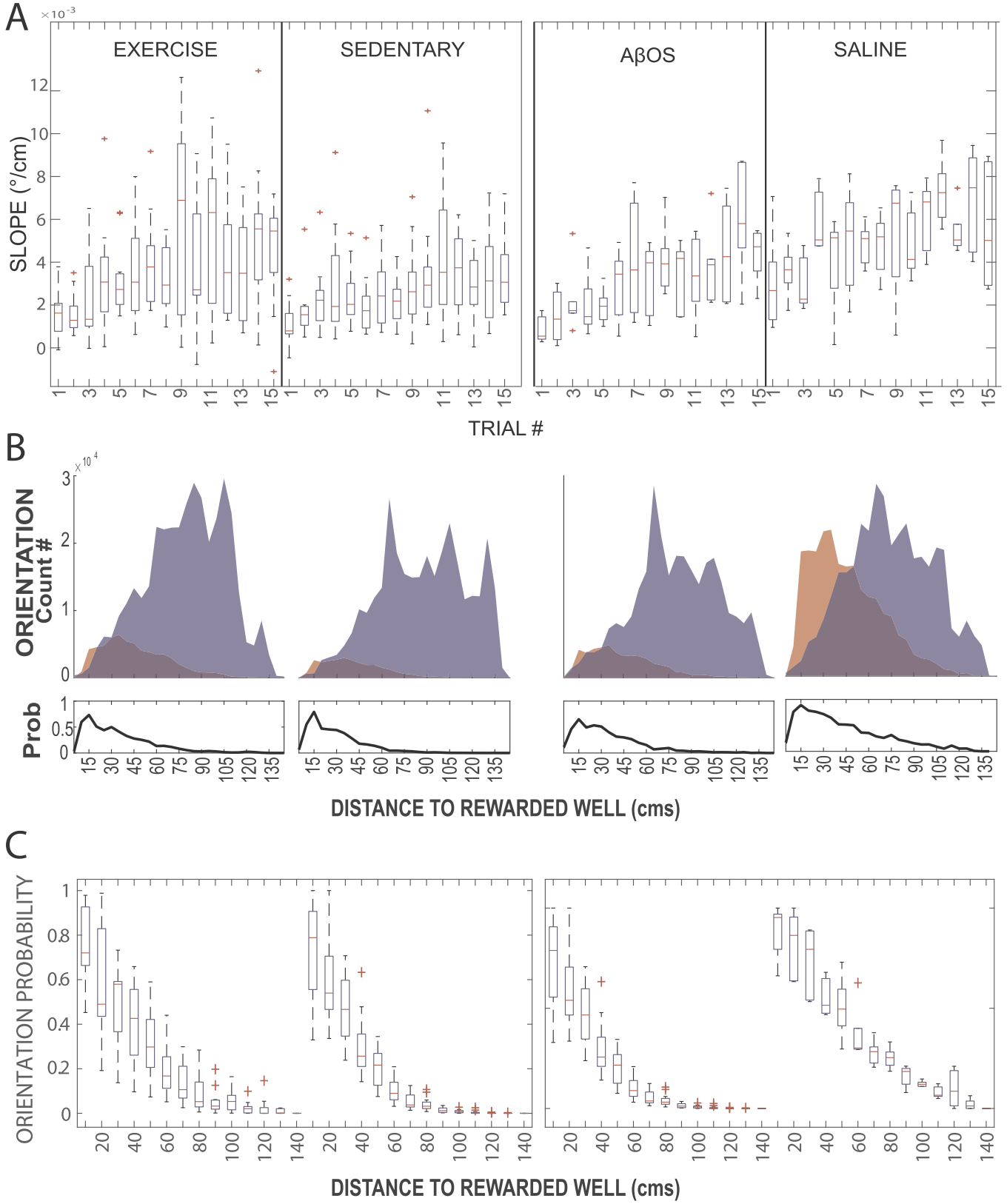

Fig. 4. Difference in navigational abilities between experimental groups. A) Orientation slope across trials and between experimental groups. Each panel depicts boxplots of the slope calculated as on Fig. 3C, but now separating the progression of the slope across trials by experimental condition. B) Each panel represents the histogram of all animal positions according to their distance to the rewarded well. The distances corresponding to the last 4 s of the trajectory are indicated in orange and the remaining are in purple. The probability under each panel corresponds to the ratio between the count in orange among the total (orange plus purple). This number represents the proportion of cases where the rat found the reward in the lasting 4 s given a distance to the rewarded well. C) Same probability as on B but estimated for each animal.

slope is indicated for each group. Two-way ANOVA with factor trial number and animal group show significant difference for trials (d.f. = 14, F = 7.88, p = 3.52×10^{-15}) and group (d.f. = 3, F = 22.5, p = 1.11×10^{-13}) with no interaction.

To complement this analysis, we estimated the probability to locate the rewarded well at a given distance. To do this, we computed the number of distances at which the animal found the rewarded well during the lasting four-second or less. In Fig. 4B the number of distances where the animals found the reward is indicated in orange and those distances where the animal did not find the reward in purple. Using this approach is possible to estimate the probability of finding the reward in the following 4 s at different distances, as can be observed in the lower panel of Fig. 4B. As can be expected at the shortest distance the higher probability to find the reward. This analysis was conducted by using different times from 1:10 s showing that after 3 s the results remain similar (Supplementary Fig. 4).

To quantify the difference between groups we estimated the same probability for each animal and group. Fig. 4C indicated the changes in the probability across distance and groups. Two-way ANOVA for factor distance and group was performed between exercise and sedentary groups, and between AβOs and Saline groups. There is a significant effect of both distance (d.f. = 13, F = 53.32, p = 2.28 10^{-44}) and group (d.f. = 1, F = 51.36, p = 6.026 10^{-11}) between AβOs and Saline. When comparing exercise and sedentary there is a similar but weaker effect for group (d.f. = 1, F = 9.9958, p = 0.0017) and a much stronger distance effect (d.f. = 13, F = 108.1708, p = 1.61 10^{-100}). Neither of the experimental conditions showed group x distance interaction. This analysis shows, that despite that both exercises and Aβos have an effect over latencies, these experimental conditions do not alter navigation in the same manner. While the exercise group shows a similar pattern of orientation probabilities than sedentary, the AβOs intervention strongly alters the capability of animals of orienting at longer distances compared with saline-treated rats. In fact, when comparing the probability of orientation at each distance between exercise and sedentary group, only non-significant differences (maximum t-value among al comparisons is t = 0.271 with a p-value of 0. 96) were detected. On the other hand, when comparing AβOs and saline, the latter showed significantly greater probabilities at already 40 cms (d.f. = 7, t = -4.67 p = 0.016 Šidák corrected).

4. Discussion

Even though several spatial memory tasks is currently available [3–10] and all these tasks are sensitive or equivalent to detect hippocampal depend spatial memory [6], each of them has clear advantages or disadvantages. Water mazes are dependent on stressful condition or negative reinforcement [34,35], this maze is not similarly executed by rats or mice, while dryland mazes do not show differences between rodent species [36,37]. Dryland mazes, such as the Barnes maze is also negatively reinforced with aversive stimuli such as bright light [4]. The radial maze has a limited option of possible routes or strategies to be solved as has been discussed [8]. The ziggurat offers a more ecological environment since gives to the animal different spatial perspective to solve the maze, but requires a long schedule of food restriction to enhance motivation and a long training period given the complexity of the task [8]. The original version of the Oasis maze [7,38] consisted of 400 equidistant wells over an open field arena. This high number of possibilities makes it hard for the animal to solve it. For this reason, this present version of the maze was modified only to contain 21 wells equidistantly distributed on the arena. This modification reduces the number of possibilities while retaining enough difficulty to test spatial memory and behavioral strategies. This version of the Oasis maze was sensitive to detected hippocampal dysfunction in rats treated with AβOs as we reported before (More tel 2018) or even in mice [39]. Also, this task was sensitive enough to detect memory-enhancing as was observed in exercise versus sedentary group (present work). In addition it has been suggested that tasks that used open field arena are susceptible to display anxiety-like behavior, nevertheless in this present version of the Oasis maze this variable could be detected, analyzed and controlled by a more extended pretraining period as was indicated before. Then this modifies Oasis maze give us the possibility to better study different behavioral features of the spatial navigation during learning and reduce confounding factor such as stress, task complexity or other.

Our Factor analysis conducted in different animal groups indicated that the three first factors (Learning, angular variability, and speediness) could explain the 65.5 % of the total explained variance, these three factors are the ones with the most noticeable size effects over trials and group based on ANOVA's analysis. The first factor, learning could efficiently discriminate differences across trials as a clear indicator of learning progression but, is a weak indicator of animal group differences. Among the variables that compose this first factor, the path length is the most prominent, suggesting that this variable must be prioritized besides other to test spatial learning

The second factor, angular variability, could efficiently discriminate differences between experimental conditions but, is a weak indicator of learning progression. The variable with the highest weight into this second factor was the standard deviations of the angle to the target, suggesting that this variable must be prioritized besides other to discriminate animal groups. Interesting straightness is a variable present in factors 1 and 2, and it could be a good indicator of both learning progression and group differences.

The third factor, *speediness* is an indicator of learning progression, however, the effects are only seen during the first trials and do not show a clear progression during a learning session. This finding suggest that it is possible that the learning process has two different steps or components which have different timing, where a significant change in behavior could occur during the first few trials of learning in a discrete mode while other changes in behavior are improving in more progressive or continues way, as can be observed in latency or straightness variables.

The rest of the total explained variance was explained by another four factors were each of them contributed in minor proportion, compared with the previous ones, with smaller effects size or event non-significant. Among these factors, the one with higher explained variance was the fourth factor which included variables such as distance to center and distance to border. All these variables are usually related to anxiety level, as it is reported on open field arena test [40], were highly anxious animal tends to walk around the border of the maze (thigmotaxic). This anxiety component was lower in those experimental animal groups that were under a more extensive pretraining process (before and after surgery), such as the AβOs and Saline-injected animals suggesting that this anxiety component could be reduced with the proper pretraining schedule. This factor analysis indicates that differences between groups could not only be explained by changes in latency or navigation speed but rather these differences could also be explained by different navigational strategies which are susceptible to be analyzed by a precise description of angular variables

Our factor analysis results are in agreement with the previous report in the Morris water maze [22], were variables such as path length, target zone time, straightens, border time and latency, are included as the major contributor among factors. Similar to our analysis, the first factor observed by Wolfer and Lipp [22] contains latency and path length as the main variables. Furthermore, our analysis has two additional contributions to detected animal group differences and progression learning. First, it includes other factors and variables, especially those that aim to reveal spatial orientation processes such as angle to the target or turning angle. This set of new variables were grouped in a new second factor which gives us additional information about the spatial navigation process. These variables were not contained in our first factor, which can be compared to the first factor found by Wolfer analysis [22]. These results indicated that this second factor is not just a different way to measure the same learning process. This second factor

ANOVA's analysis demonstrated a better sensitivity to detected animals group differences, and not learning as the first factor. Second, we complemented the factor analysis, with ANOVA´s comparison which able us to determine differences in the learning process or animal's experimental groups and provided some guidelines to interpret each factor accurately.

The problem of interpreting the meaning of those factors or latent variables is a well-known problem in factor analysis. Graziano et al. [20] addressed this problem by classifying the animal behavior in several qualitative categories. Then they performed a discriminant analysis over a comparable list of variables. In the present work, instead of this qualitative categorization, which requires the visual inspection of the animal behavior by the experimenter, our analysis does not make any a priori categorization. Instead, we base our interpretation on both factor analysis, linear regression and ANOVAs which are all of them independent of the observer expertise.

The present work also analyzed navigation strategies used to solve the task, since several navigational strategies may be used by animals. For example, animals can get oriented by different taxis mechanisms, such as the presence of sensory clues (images, odors or sounds) on the target that prompt the animals to get oriented to those sources of stimuli [31]. A second possibility is that the animal uses mnemonic non-spatial elements such as procedural strategies where the animal develops a stereotyped sequence of movements to arrive at the target. A third possibility is that the animals use their internal spatial navigational system to represent the environment and the target location. Under this strategy animals must be able to update its internal representation of the maze to make it consistent with the available sensory information, guiding its behavior toward the target. Because in principle animals may use any of these strategies, analyzed which was the principal mechanism used by the animal to solve the maze. The two first options (sensory-guided taxis or procedural components) could be discarded by an experimental setting. First, the target zone was indistinguishable from another 20 possible non-reward wells and water was used as a reward to prevent olfactory clues, which means the target zone is not salient compared with other zones in the maze. Second, to prevent procedural strategies during the task execution, the animals start from a different position in the maze on each trial, forcing the animals to reoriented in the maze each time. Also, the step correlation and angle/distance analysis demonstrated that the animals do not show any procedural behavior to solve the maze since the animals do not make a stereotyped movement, but, conversely, the animals correct their path-direction to the target zone, at each step, independently of the starting position. Regarding sensory-guided taxis, even though the physical attributes of the target are controlled, it may be possible that the animals still can directly locate the target by using sensory information. Nevertheless, the angle/distance analysis shows that animals can locate the target zone at farther distances throughout all the consecutive trials, indicating that the animal experiment a continuous spatial learning process. Discarding the possibility that the animals recognize the target zone just by sensory features, because once the target is recognized, no behavioral improvement should be observed in the following trials.

The previous results also contribute to our understanding of the strategy used by rodents to solve navigation using visual landmarks. It has been recently proposed that animals use sensory cues to estimate the location of a target [41], and that the learning progression partly dependent on the ability of animals to use cue information. We complement these findings, by corroborating that animals progressively have a better estimation of the target location, as we could observe an improvement in step-correlation values and greater slopes in the angular/distance regressions. It suggests that rats may abstain from going towards sensory cues at the latter stages of the learning, once the animals have a confident target estimation. In fact, we observed that the animals go straight to the target later trials. The present work also shows that this ability to locate the target can be modulated in AβOs treated animals, because they have difficulty to get oriented at longer distances.

In addition, the changes in the angle/distance across trials as a measure of orientation strategies were different from each experimental condition. This indicates that part of the cognitive improvement (exercise) or cognitive impairment (AβOs) could be explained by different animal's strategies to solve the learning task. As we observed in Fig. 4 the AβOs treated animals has a significant impairment on the capacity to get oriented, at longer distance compared with control. Exercise did not change significantly the orientation strategy; however, these animals have the shortest latencies and path distance. This highlight that latency changes could be explained by different mechanisms during navigation, for example, velocity in exercised animal or orientation capacity in AβOs treated animals. Emphasizing the need to explore a wide number of variables to be analyzed, during spatial navigation to a more comprehensive understanding of animal behavior changes during spatial learning.

5. Conclusions

In conclusion, this new version of the Oasis maze clearly detects and able us to explore learning progress, animal group differences, spatial learning strategies and non-related variables such as anxiety. Our factors analysis could able us to prioritized which variables better fit with learning or navigational processes. Also, this analysis unveils which factors are more sensitive to discriminate learning progress or which of them are more sensitive to discriminate animal group differences, and which factor may detect another confounding behavioral component such as anxiety. This PCA/ANOVA analysis suggests that learning and spatial navigation could be treated as separate dimensions of animal behavior. The analysis of navigational strategies gives us relevant information about how different animal groups navigated and get oriented and how this process could affect the animal's performance. This result suggests that typically measured variables such as latency reflex learning progression but not give any information about what is changing in the animal behavior that explains the changes in the time to solve a learning task. Our navigational strategies analysis indicates that changes in latency could be explained by changes in velocity or in spatial orientation capabilities depend on which animal conditions is has been analyzed.

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CRediT authorship contribution statement

Miguel Concha-Miranda: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - original draft. **Jamileth More:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Noemi Grinspun:** Conceptualization, Methodology, Investigation. **Cristian Sanchez:** Conceptualization, Methodology, Investigation. **Andrea Paula-Lima:** Methodology, Funding acquisition. **José L. Valdés:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing - review & editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bbr.2020.112555.

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